embryo to an advanced cleavage stage embryo;

separating and culturing the cleaved cells of the embryo.

REMARKS

In the Official Action dated August 9, 2002, the application has been objected because of alleged informalities. Claims 31 and 32 have been rejected as allegedly identical in scope. Claims 22-32 have been rejected under 35 U.S.C. § 101 as allegedly directed to non-statutory subject matter. Claims 22-32 have also been rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enabling support. Claims 22-32 have been further rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Claims 28-32 have been rejected under 35 U.S.C. 102(b) as allegedly anticipated by Campbell et al. (1996).

This response addresses each of the Examiner's objections and rejections. Accordingly, the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

The application has been objected to because of alleged informalities. Specifically, the Examiner contends that in the table on page 32, the text underneath "oocytes" is illegible. In response, Applicants attach hereto, as Exhibit A, a more legible copy of page 32. No new matter has been added.

The Examiner contends that the recitation "animals" at page 30, line 18, and page 31, line 14 in Example 12, should indicate what animal species used. In response, Applicants have amended the specification to replace "animals" with "rats" at page 30, line 18, and page 31, line 14 in Example 12. No new matter has been added.

The Examiner further contends that there is no guidance regarding what was used to transfect the fibroblasts in the table on page 32. Applicants respectfully direct the Examiner's attention to the specification from page 16, line 28 to page 17, line 4, which provides guidance regarding the use of a construct comprising a lacZ gene to transfect the fibroblasts.

Accordingly, each of the Examiner's objections is overcome, and withdrawal thereof is respectfully requested.

Claims 31 and 32 have been rejected as allegedly identical in scope. In response, Applicants have cancelled Claims 22-32, without prejudice. Accordingly, the rejection of Claims 31-32 is rendered moot and withdrawal thereof is respectfully requested.

Claims 22-32 have been rejected under 35 U.S.C. § 101 as allegedly directed to non-statutory subject matter. Specifically, the Examiner contends claims encompass human beings and methods of making human beings which are non-statutory subject matter. In response, Applicants have cancelled Claims 22-32, without prejudice. Accordingly, the rejection of Claims 22-32, under 35 U.S.C. § 101, is rendered moot, and withdrawal thereof is respectfully requested.

Claims 22-32 have also been rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enabling support. Specifically, the Examiner contends that the specification fails to provide an enabling disclosure for animals of the type claimed because methods of nuclear transfer are not routinely successful. More specifically, the claims have been rejected for reciting "introducing a nucleus into an oocyte or embryo", as embryos are allegedly not used for nuclear transfer.

In the first instance, Applicants respectfully submit that, in an effort to expedite favorable prosecution, Claims 22-32 have been cancelled, without prejudice. New Claims 45-66

have been added. Support for the new claims can be found throughout the specification and in claims 22-32, as originally filed. No new matter has been added.

It is submitted that the present invention provides the first use of neural stem cells (NSC) for cloning purposes, as conceded by the Examiner.

It is submitted that the protocols used to isolate and propagate NSC are essentially the same in all mammalian species from which neural stem cells have thus far been isolated, including humans, cattle, pigs, rats and mice. Support for such protocols can be found throughout the specification and specifically in the Example, on page 23, lines 15-18, for example. Therefore, the technology is applicable to all animals. While rats have been specifically exemplified in the specification, applicants submit that restriction of the present invention to rats is neither warranted nor required.

Applicants further submit that a basis of the disclosed protocol is the disaggregation of a piece of central nervous tissue isolated from the brain of either a fetus or an adult, and culturing the resulting cell suspension in a basal media containing little or no serum and at least one of the growth factors, e.g. bFGF and EGF. In accordance with the present teachings, this protocol can be employed across all mammalian species. The neural stem cells so isolated show similar characteristics across species, e.g., potential to differentiate into all neural tissue, propensity to grow in spheres in culture, ability to transdifferentiate into different tissue types such as muscle and blood.

In addition, the NSC may be any form of NSC, preferably fetal NSC (FNSC) or FNSC that have been genetically modified with telomerase catalytic component (TERT) to provide such cells with the capacity of continuous growth. Similarly, the basic techniques for nuclear transfer are the same across all mammalian species and require the same technical skill

and equipment. The oocytes are obtained from the female donor (flushed from the animal following superovulation, natural ovulation or isolated from the ovaries of animals and matured *in vitro),* enucleated using micromanipulation or oocyte bisection and then the donor nucleus is introduced into the oocyte by microinjection or fusion of the enucleated oocyte with the donor cell. Development of the reconstructed embryo is either triggered chemically or following an electric pulse. The activated embryo may then be cultured *in vitro* for several days before being transferred to a recipient animal or transferred immediately to a recipient animal and allowed to develop to term.

Moreover, it is submitted that the main differences between species are the size of the oocyte and the time periods in which the embryos develop. The former of these requires an adjustment of the parameters used, e.g., in the size of the needles used to enucleate the oocytes or the strength of the electric pulse used to activate development, but not a material change in the protocol. The latter relates more to the timing of transfer of the resulting embryo to the recipient animal and the time taken for the embryo to develop to term to produce an animal; it does not affect the nuclear transfer procedures per se. Differences also exist between species in the ability and efficiency with which embryos can be cultured *in vitro*; however, this can be overcome by transferring the reconstructed embryos directly to recipient animals as opposed to culturing them for a period *in vitro* before transfer.

Furthermore, Applicants submit that the detailed disclosure provided in the present application describes that the use of neural stem cells in nuclear transfer procedures in the rat can readily be replicated in other species by those skilled in the art, with little or no variance from the basic methodologies described. Similarly, the basis for using neural stem cells as the nuclear donor in nuclear transfer techniques, i.e., the use of a donor nucleus from a cell type that

has the potential to develop into a range of tissues, is valid across all mammals, not just rats. Hence, the description and enablement of the technology in one mammalian species by the present invention, serves as validation of the approach in all mammalian species.

The Examiner acknowledges that 78 embryos have been provided having nuclei from neural stem cells in Example 13. Applicants confirm that these embryos resulted from nuclear transfer techniques in rats using neural stem cell nuclei as donors. These embryos were successfully transferred to recipient rats.

The Examiner's further contends that the recitation "an oocyte or embryo" lacks enablement because embryos are not used for nuclear transfer. In an effort to expedite favorable prosecution, Applicants have cancelled Claims 22-32. Newly added Claims 45-66 do not recite "embryos" for nuclear transfer.

In view of the foregoing, the rejection of Claims 22-32 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enabling support, is overcome. Withdrawal of the rejection is respectfully requested.

Claims 22-32 have been further rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite.

Specifically, the Examiner contends that Claims 22-32 are indefinite by reciting "a continuously growing donor cell nucleus." The Examiner also contends that Claims 22-32 are unclear by reciting "a continuously growing donor cell." The Examiner further contends that Claims 22-32 are indefinite in the recitations of "[a] method of producing an animal" and "to preferably develop to a fetus or animal". The Examiner also contends that Claims 23-24 are indefinite by reciting "a continuous growing somatic cell."

In response, and in an effort to expedite a favorable prosecution, Applicants have cancelled Claims 22-32. Added Claims 45-66 do not contain the rejected recitations. Applicants further submit that the recitation "continuously growing cells" has been clarified as "cells capable of long-term culture" in the added claims. Support for this recitation is found throughout the specification and particularly in Example 7. No new matter has been added.

The Examiner contends that Claims 24 and 27 are indefinite in their recitation of "destroying" and "deleting" as it is allegedly unclear how "destroying" would be distinguished from "deleting."

It is submitted that the difference between these terms is that a destroyed gene need not be deleted and removed. A "destroyed" or disrupted gene may be inactivated but remains within the DNA sequence. A "deleted" gene is clearly one that has been removed. Support for these terms can be found throughout the specification and particularly at page 6, lines 15-21, page 23, lines 1-5, for example. In addition, the definition of such terms are commonly used by textbooks and popular reference books, such as Wu, et al., *Methods in Gene Biotechnology*, CRC Press, 1997, on pages 343-344, for example.

The Examiner contends Claims 24 and 27 are indefinite by reciting "said genetic modification". In response, and in an effort to expedite a favorable prosecution, Claims 24 and 27 have been deleted.

The Examiner contends that Claims 26-27 are infinite by reciting "TERT cell" as the term allegedly lacks a definition. In response, Applicants respectfully direct the Examiner's attention to the specification, on page 10 Am2 1, first paragraph, where the term "TERT cell(s)" is specifically defined. The TERT component provides continuous growth to the cell. The term

TERT is specifically defined by the specification as a telomerase catalytic component. See, for example, page 9, line 23.

The Examiner contends that Claims 29-30 are indefinite as the phrase "optionally" is allegedly confusing. In response, the term "optionally" has been deleted from the added claim set.

In view of the foregoing, the rejection of Claims 22-32, under 35 U.S.C. § 112, second paragraph, as allegedly indefinite, is overcome. Withdrawal of the rejection is, therefore, respectfully requested.

Claims 28-32 have been rejected under 35 U.S.C. 102(b) as allegedly anticipated by Campbell et al. (1996). Specifically, the Examiner contends that Claims 28 and 30-32 are product-by-process claims. The Examiner alleges that sheep produced by nuclear transfer, the cell line, TNT4, used as the nuclear donor cell, and the reconstructed sheep embryos, disclosed by Campbell et al., would not be structurally different from the animals claimed in Claims 31-32, a cell line as claimed in Claim 30, and an embryo as claimed in Claim 28. Moreover, the Examiner contends that Claim 29 is anticipated by the methods disclosed in Campbell, et al., for producing a cell line from an embryo isolated from sheep.

In response, Applicants have cancelled Claims 28- 32, without prejudice. Claim 29 has been rewritten as Claim 64 depending on Claim 45 which relates to producing a genetically modified embryo using NSC, as presently claimed. Campbell et al. do not teach or disclose a method of producing a genetically modified embryo using NSC. Therefore, the rejection of Claims 28-32, under 35 U.S.C. § 102(b), is overcome. Withdrawal of the rejection is respectfully requested.

Claims 29 and 30 have also been rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Evens and Kaufman (1981). Specifically, the Examiner states that the Evans and Kaufman reference discloses a method for producing a cell line from a mouse embryo.

As indicated above, Claims 29-30 have been cancelled, without prejudice. Applicants submit that the methods of producing the cell line by obtaining an embryo produced by the methods of Claims 45 and 62, relate to an embryo produced by a nuclear transfer method utilizing an NSC or a genetically modified NSC which is simply not taught by the Evans and Kaufman.

Accordingly, Applicants respectfully submit that the rejection of Claims 29-30 under 35 U.S.C. §102(b), is overcome. Withdrawal of the rejection is respectfully requested.

Attached hereto is a marked-up version of the changes made to the specification and claims by the instant amendment, captioned "**Version with Markings to Show Changes Made.**"

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

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Encl.: Version with Markings to Show Changes Made
Exhibit A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The paragraph beginning at line 18, page 30 has been amended as follows:

[Animals]Rats were killed by decapitation and the oviducts removed in less than 5 minutes. Oviducts were collected into prewarmed calcium free phosphate buffered saline (PBS). Oocytes were liberated from the oviducts into M16 culture medium containing 40 IU/ml hyaluronidase at 37 °C using fine forceps. Oocytes were washed twice in M2 medium after 5 minutes exposure to hyaluronidase. Cumulus free oocytes were transferred to equilibrated modified rat embryo culture medium (MR1ECM) and incubated in humidified 5 % CO_2 in air at 37 °C until use. Oocytes at the metaphase II state (i.e. with the first polar body extruded) were selected for nuclear transfer (NT).

The paragraph beginning at line 14, page 31 has been amended as follows:

Embryos were transferred back to primed recipient [animals]rats on day 2, 3 or day 4 of culture.

In the Claims:

Claims 22-32 have been canceled without prejudice.

The following claims have been added:

45. A method of producing a non-human embryo, said method comprising introducing a nucleus from a neural stem cell (NSC) into an oocyte and allowing the oocyte to mature to the non-human embryo.

46. The method according to claim 45 wherein the NSC is a fetal NSC (FNSC).

47. The method according to claim 45 wherein the NSC is a telomerase catalytic component

(TERT) NSC.

48. The method according to claim 45 wherein the NSC is a telomerase catalytic component (TERT) FNSC.

49. The method according to claim 45 wherein the NSC is capable of long term culture and is derived from a cellular composition prepared by a method comprising:

obtaining a source of neural stem cells;

preparing a suspension of cells from the source;

contacting the suspension of cells with a suitable medium to maintain the neural stem cells in a long term cell culture; and

culturing the cells in the long term culture, wherein said culturing comprises passaging and propagation of the cells.

50. The method according to claim 49 wherein the long term culture is a period of 4 to 6 weeks.

51. The method according to claim 49 wherein the source of the neural stem cell is a fetus differentiated at a stage after the embryonic stage.

52. The method according to claim 51 wherein the source of the neural stem cell is a head or spinal cord of the fetus.

53. The method according to claim 49 wherein the suitable medium includes at least one lipid and at least one mitogenic factor.

54. The method according to claim 53 wherein the lipid is selected from the group consisting of cholesterol, triglycerides or phospholipids or a combination thereof.

55. The method according to claim 53 wherein the mitogenic factor is selected from the group consisting of bFGF, EGF, PDGF or a combination of EGF and bFGF.

56. The method according to claim 55 wherein the EGF is in the range of 2 to 20 ng/ml.

57. The method according to claim 55 wherein the bFGF is in the range of 2 to 20 μ/ml.

58. The method according to claim 53 wherein a chemically defined lipid concentrate is present in a ratio of 1:100.

59. The method according to claim 53 wherein the media further includes a cell survival factor.

60. The method according to claim 59 wherein the cell survival factor is selected from the group consisting of transferrin, insulin, growth factors including EGF, bFGF (FGF-2) or PDGF, lipids and selenium.

61. The method according to claim 49 wherein the passaging and propagation of the cells is conducted when the cells bud from the cell culture.

62. The method according to claim 45 wherein the NSC is genetically modified and wherein the genetic modification comprises destroying, modifying or deleting a gene.

63. A method of producing a genetically modified non-human animal said method comprising:

obtaining an embryo prepared by the method according to claim 45; and allowing the embryo to mature to the genetically modified non-human animal.

64. A method of producing a genetically modified non-human animal said method comprising:

obtaining an embryo prepared by the method according to claim 62; and allowing the embryo to mature to the genetically modified non-human animal.

65. A method of producing a cell line from an embryo to produce cloned cells of an embryo, said method comprising:

obtaining an embryo prepared by the method according to claim 45; culturing the embryo to an advanced cleavage stage embryo; and

separating and culturing the cleaved cells of the embryo.

66. A method of producing a cell line from an embryo to produce cloned cells of an embryo, said

method comprising:

obtaining an embryo prepared by the method according to claim 62; culturing the embryo to an advanced cleavage stage embryo;

separating and culturing the cleaved cells of the embryo.

Example 13. Results from nuclear transfer experiments using transfected fibroblasts and FNS cells.

Methods for nuclear transfer of fibroblast or FNS cell nuclei are as detailed in Example 12

	Donor Cell Type	
	Transfected Embryonic Fibroblast	Neural stem cells
Oocytes	(%)	(%)
oocytes undergoing nuclear transfer	1256	317
Survived transfer	106 (8.4)[b]	80 (30.5)[b]
Cleaved to 2-cell embryo	24 (22.6)[b]	N/A
Embryos		
Transferred to Mice	7	nil
Transferred to Rats	nil	78
Developing to Morula/Blastocyst	1 (14.3)[a]	nd
Producing Live Born	nd	0

Significant differences in reconstructed embryo survival, cleavage and development *in vivo* between donor cell types are indicated by different superscript letters (a-b). Relative percentages surviving each manipulation are shown in parentheses. nd: not determined.

Finally, it is to be understood that various other modifications and/or alterations may be made without departing from the spirit of the present invention as outlined herein.